Dynamics of some carbon and nitrogen metabolism enzymes during the day in various wheat genotypes under drought

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PEPC plays a pivotal role in various metabolic processes in C_3 plants such as providing intermediates for Krebs cycle, maintaining intracellular pH and osmotic pressure, regulation of the movement of stomatal guard cells, refixation of CO_2 formed by respiration, forming carbon skeleton for the lipid synthesis during the grain development period and nitrogen assimilation. Aspartate aminotransferase is essential in the primary nitrogen assimilation, transportation of the reducing equivalents, exchange of carbon and nitrogen resources among cellular subcompartments. Durum (Barakatli 95 and Garagylchyg 2) and bread wheat genotypes (Gobustan and Tale 38) cultivated in the experimental field of the Research Institute of Crop Husbandry were used as the study materials. The high activities of PEPC and NAD-MDH during the morning hours and a positive correlation existing between them during the day suggest that functioning mutually, these enzymes participate in the biosynthesis of malic acid.

Keywords: *PEP-carboxylase, aspartate aminotransferase, NAD-malate dehydrogenase, carbon metabolism, nitrogen metabolism, daytime*

INTRODUCTION

Annual production of wheat, which meets one-fourth of the world population demand in protein and calorie, is ~700 million tons. This crop is cultivated in 215 million ha area, which is more than 16% of the world sowing fields. The main goals of the modern and future biotechnology are production of high-quality bread and biofuel, creation of wheat varieties convenient for the human consumption (http://faostat.fao.org).

Playing a crucial role in carbon and nitrogen metabolism of plants, PEPC catalyzes irreversible carboxylation of phosphoenole pyruvate (PEP) and converts it into oxaloacetate (OAA) (Huppe et al., 1994). The conducted studies are still not sufficient to elucidate the physiological role and regulation mechanisms of C_3 PEPCs (O'Leary et al., 2009). Currently, the role of PEPC in carbon and nitrogen metabolism of plants has not been studied sufficiently under *in vivo* conditions.

Aspartate aminotransferase (AsAT, EC 2.6.1.1) plays an important role in primary nitrogen assimilation, transportation of reducing equivalents and exchange of carbon and nitrogen resources among cellular subcompartments (Gaufichon et al., 2016). Several isoforms of AsAT are localized in subcellular organoids – cytosol, chloroplasts, mitochondria and peroxisomes - of plants (Duff et al., 2011).

NAD-malate dehydrogenase (NAD-MDH, EC 1.1.1.37) participates in some metabolic processes, including tricarboxylic acid cycle and glyoxylate cycle, synthesis of aminoacids, glyconeogenesis and exchange of meatbolites between cytosol and subcellular organoids (Nunes-Nesi et al., 2005; Schertl, Braun, 2014; Scheibe, 2004).

It is important that plants can maintain a correct N:C ratio, and to achieve this, various biochemical processes have developed. These processes enable the plant to adjust its metabolism and accommodate environmental stress conditions

(Coruzzi & Zhou, 2001).

The aim of the present work was to investigate the diurnal changes in the activities of PEP, ASAT and NAD-MDH in flag leaves of drought-tolerant and drought-sensitive wheat genotypes cultivated under irrigated and rainfed conditions in the field.

MATERIALS AND METHODS

Durum (Barakatli 95 and Garagylchyg 2) and bread wheat genotypes (Gobustan and Tale 38) cultivated in the experimental field of the Research Institute of Crop Husbandry located in the Absheron peninsula were used as the study materials. Samples were taken at three-hour intervals. The plant material was ground in the presence of quarts sand using the cooled mortar and pestle. 100 mM Tris-HCl buffer used for the homogenization contained 10 мM $MgCl_2$, 1мM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol (DTT) , 2 mM phenylmethanesul-fonyl fluoride (PMSF) and 2% (w/v) insoluble polyvinylpyrrolidone(PVP).

PEPC activity was determined in 1 ml reaction medium containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2mM DTT, 10mM $NaHCO_3$, 0.2 mM NADH, 10U/ml MDH, 10 mM PEP and 40µl enzymatic extract. The reaction was initiated by adding the substrate (10 mM PEP) to the reaction medium (Pyankov, 2000).

AsAT activity was measured in the reaction medium containing 100 mM HEPES-KOH (pH 7.4) and 100 mM Tris-HCl (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutorate , 10µg/ml pyrodoxalphosphate, 10 mM DTT, 12 U/ml MDH, 0.2 mM NADH, 20µl leaf extract and 2.5 mM L-aspartate. The reaction started by adding L-aspartate (Alfonso & Brüggemann, 2012).

NAD-malate dehydrogenase activity was determined by adding the substrate (1 mM oxaloacetate) to the reaction medium containing 100 mM Tris-HCl (pH 9.0), 30 mM malate and 0.2 mM NAD.

The enzyme activities were determined using the spectrophotometric method (Ultrospec 3300 Pro, Amersham, USA). The measurements were performed in 1ml cuvette, at 340 nm wavelength (Scheibe & Stitt, 1988). .

Total protein assay: Total soluble protein was determined spectrophotometrically, using 0.12% Coomassie Brilliant Blue G-250 (Sedmak & Grossberg, 1977).

RESULTS AND DISCUSSION

Phosphoenolpyruvate carboxylase, aspartate aminotransferase and NAD-malate dehydrogenase activities were studied comparatively in durum and bread wheat genotypes with contrasting drought tolerance. In both variants of the durum wheat genotypes (Barakatli 95 and Garagylchyg 2) the highest PEPC activity was observed at 8^{00}. Whereas, in the bread wheat genotypes (Gobustan and Tale 38) the highest PEPC activity was found at 17^{00} in flag leaves. There was no significant changes in the enzyme activity during the morning and afternoon hours(Figure 1).

The highest AsAT activity was registered in the watered variant of the Barakatli 95 variety, the lowest activity in flag leaves of the stressed Garagylchyg 2 variety, which is drought sensitive (Figure 2).

The maximum activity of AsAT was observed in flag leaves of both variants of the drought-tolerant Gobustan variety at 8^{00}. At 11^{00} the enzyme activity declined 1.3 and 1.5 times in watered and drought-exposed plant leaves, respectively. The AsAT activity decreased gradually during the day and began to increase from 17^{00}, reaching the values observed in the morning hours. Performing transamination in various cell compartments, AsAT catalyzes the reaction of formation of aspartate and 2-oxoglutaric acid from glutamate and oxaloacetate. AsAT plays an important role in primary nitrogen assimilation, transportation of reducing equivalents, exchange of carbon and nitrogen resources among cellular subcompartments.

The maximum NAD-MDH activity was found in all samples taken from Barakatli 95 at 17^{00} with the exception of the drought-exposed variant. In the bread wheat varieties the highest NAD-MDH activity was found in the morning hours (8^{00} and 11^{00}) of the day (Figure 3). A gradual decrease in NAD-MDH activity was observed in flag leaves of the drought-exposed Gobustan variety and watered Tale 38 variety during the day. In leaves of C_3 plants malate displays a diurnal rhythm.

Increased during the light period, the enzyme level reached the maximum value to the end of the day and decreased during the night. Malate synthesis during the light period is the result of the sequential functioning of PEPC and MDH. Thus, acting as a primary carboxylating enzyme, PEPC combines CO_2 with PEP and forms 4-carbon, dibasic oxaloacetic acid.

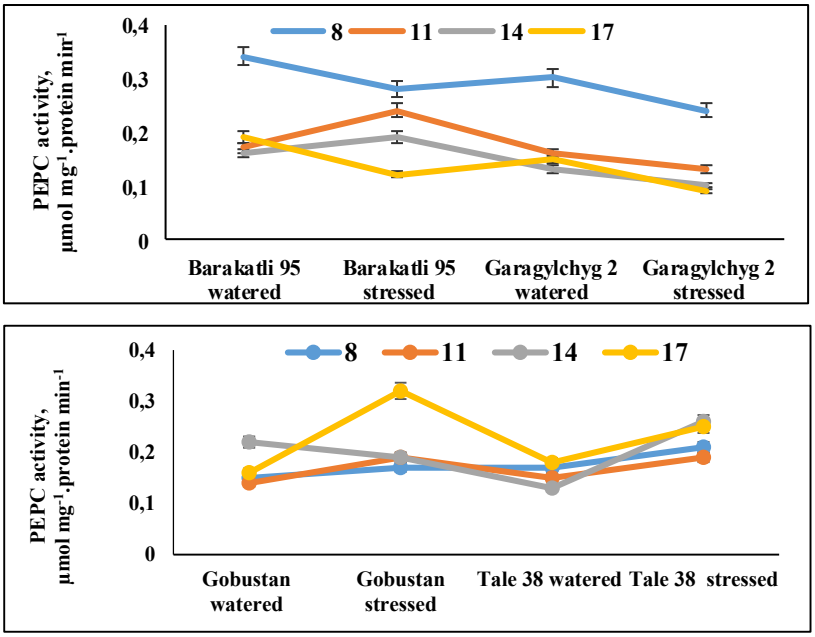

Fig. 1. Dynamics of PEPC activity in flag leaves of durum and bread wheat genotypes during light phases of the day.

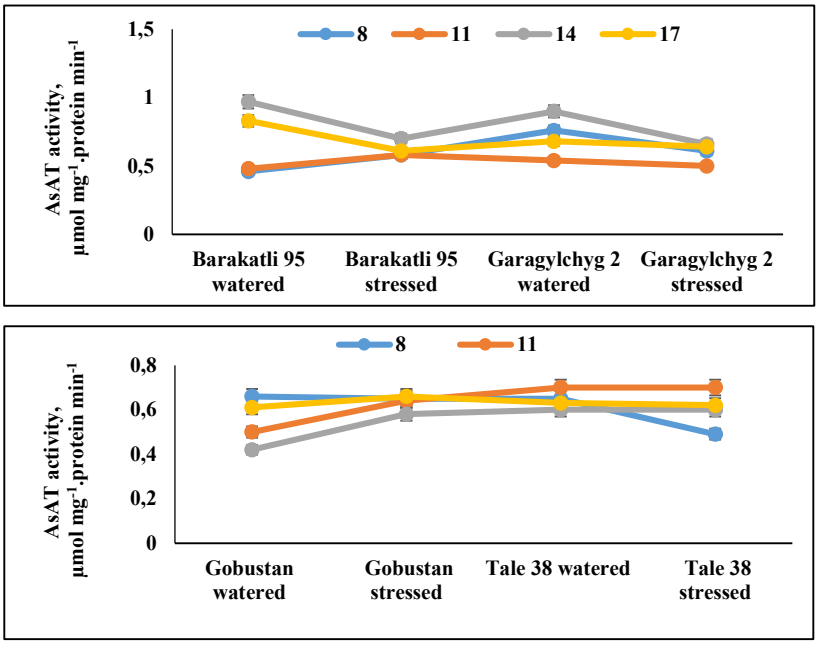

Fig. 2. Dynamics of AsAT activity in flag leaves of durum and bread wheat genotypes during light phases of the day.

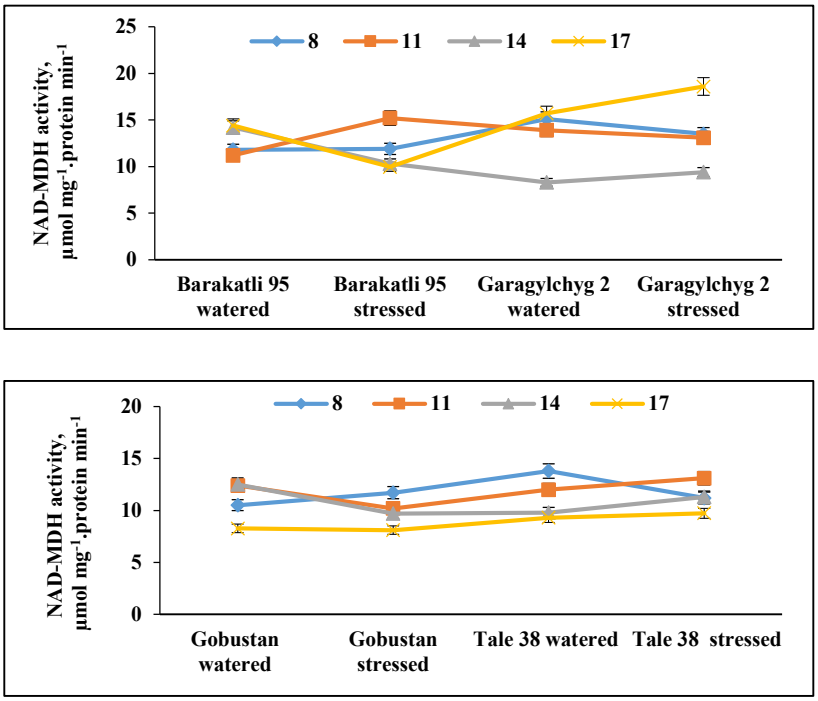

Fig. 3. Dynamics of NAD-MDH activity in flag leaves of durum and bread wheat genotypes during light phases of the day.

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Müxtəlif buğda genotiplərində karbon və azot metabolizminin bəzi fermentlərinin quraqlıq stresinin təsirindən gün ərzində dəyişmə dinamikası

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C_3 bitkilərdə FEP-karboksilaza Krebs tsiklini aralıq birləşmələrlə təmin etmək, hüceyrə daxili pH-ı və osmotik təzyiqi saxlamaq, ağızcıqların qoruyucu hüceyrələrinin hərəkətini tənzimləmək, tənəffüs zamanı əmələ gələn CO_2 qazını yenidən refiksasiya etmək, toxumun inkişafı dövründə lipid sintezi üçün karbon skeletlərinin yaradılması və azotun assimilyasiyası kimi müxtəlif metabolik proseslərdə mühüm rol oynayır. Aspartataminotransferaza ilkin azot assimlyasiyasında, reduksiyaedici ekvivalentlərin nəqlində və hüceyrə subkompartmentləri arasında karbon və azot ehtiyatının qarşılıqlı mübadiləsində əsas rol oynayır. Tədqiqatın materialları bərk buğda genotipləri (Bərəkətli-95 və Qaraqılçıq-2) və yumşaq buğda genotipləri (Qobustan və Tale-38) Elmi Tədqiqat Əkinçilik Institutunun Abşeron yarımadasında yerləşən təcrübə sahəsindən götürülmüşdür. Səhər saatlarında FEPK və NAD-MDH fermentinin aktivliklərinin yüksək olması və onların aktivliklərinin gün ərzində dəyişməsində əksər hallarda müsbət korelyasiyanın müşahidə olunması hər iki fermentin qarşılıqlı işləyərək malat turşusunun biosintezində iştirak etdiyini söyləməyə əsas verir.

Açar sözlər: *FEP-karboksilaza, aspartataminotransferaza, NAD-malatdehidrogenaza, karbon metabolizmi, azot metabolizmi, gündüz*